Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 2036–2040, March 1995
Plant Biology

β-Glucosidase: An elicitor of herbivore-induced plant odor that attracts host-searching parasitic wasps

(plant defense/chemical ecology/beneficial insects/tritrophic interactions/behavior)

LETIZIA MATTIACCI*, MARCEL DICKE*†, MAARTEN A. POSTHUMUS‡

*Department of Entomology, Wageningen Agricultural University, P.O. Box 8031, 6700 EH Wageningen, The Netherlands; and ‡Department of Organic Chemistry, Wageningen Agricultural University, Dreijenplein 8, 6703 HB Wageningen, The Netherlands

Communicated by Andre T. Jagendorf, Cornell University, Ithaca, NY, November 14, 1994 (received for review July 19, 1994)

ABSTRACT **Cabbage plants respond to caterpillar (*Pieris brassicae*) herbivory by releasing a mixture of volatiles that makes them highly attractive to parasitic wasps (*Cotesia glomerata*) that attack the herbivores. Cabbage leaves that are artificially damaged and subsequently treated with gut regurgitant of *P. brassicae* caterpillars release a volatile blend similar to that of herbivore-damaged plants. We demonstrate the presence of β-glucosidase in *P. brassicae* regurgitant. Leaves treated with commercial β-glucosidase (from almonds) release a volatile blend similar to that of leaves treated with *P. brassicae* regurgitant. In a flight bioassay, leaves treated with almond β-glucosidase are highly attractive to the parasitic wasp *C. glomerata*. Furthermore, the wasps do not discriminate between cabbage leaves treated with almond β-glucosidase and leaves treated with larval regurgitant. β-Glucosidase was also recorded in cabbage leaf extract, but this is not as effective as caterpillar β-glucosidase in eliciting the volatile production. Caterpillars that feed on a β-glucosidase-free diet secrete the enzyme, and their regurgitant is an effective elicitor of the plant response. These findings show that β-glucosidase is a *P. brassicae*-secreted elicitor of the defense response of cabbage plants to herbivore injury, inducing the emission of volatiles that are used by parasitoids of the herbivore to locate their victims.**

It is well-known that plants may react to herbivory or to pathogen infestation by phytochemical responses (reviewed in refs. 1–3). The first step in such responses is the recognition of the attack by the plant. How plants recognize infestation of a pathogen has been intensively studied and many pathogen-derived exogenous elicitors of phytoalexins have been identified (4). In contrast, knowledge on the recognition of herbivorous arthropods by plants is scarce, being mostly restricted to the involvement of herbivore secretions (e.g., see refs. 5–7). Yet, a wealth of knowledge is available on endogenous elicitors that originate from mechanical damage (8) and on subsequent steps in the signal transduction pathway (8, 9) in responses of plants to herbivores.

A phytochemical response that has been studied in the past decade is the production of volatiles that attract carnivorous arthropods such as predators and parasitic wasps (parasitoids) that attack the herbivore (2). For instance, lima bean plants respond to infestation by the spider mite *Tetranychus urticae* by producing volatile terpenoids and methyl salicylate that attract a predator (*Phytoseiulus persimilis*) of these herbivores (2, 10–12). Recently, studies have been initiated on exogenous herbivore elicitors of such plant responses. For instance, the response of corn plants to herbivory by fall armyworm caterpillars is similar to the response to administration of caterpillar regurgitant into a mechanical wound or fed through the petiole of an intact corn leaf (13, 14). These treatments result in the production of volatile terpenoids and indole that attract the parasitoid *Cotesia marginiventris*. The exogenous elicitor in the regurgitant has not been identified. A very interesting clue on the identity of the elicitor of herbivore-induced plant odors comes from a study of the biosynthesis of two homoterpenes that are emitted by spider mite-infested lima bean and by regurgitant-treated corn plants. Boland *et al.* (15) showed that application of β-glucosidase into a mechanical wound in lima bean leaves resulted in the production of these carnivore attractants. Application of β-glucosidase onto undamaged leaves had no effect, nor did mechanical damage without application of β-glucosidase. Lipases were not effective. This suggests that β-glucosidase may be an herbivore-related elicitor. However, this cannot be concluded until the following have been demonstrated: (*i*) its existence in herbivore secretions, (*ii*) its effect on the total volatile blend emitted, and (*iii*) the attraction of carnivores toward β-glucosidase-treated leaves. There are good reasons to investigate this because glucosidases have been found in several insect orders including Lepidoptera, where they are usually reported in the caterpillar gut (16, 17).

We investigated whether β-glucosidase is an elicitor that affects the production of carnivore attractants in a system of cabbage plants, caterpillars of the large cabbage white butterfly *Pieris brassicae* and the parasitoid *Cotesia glomerata*. The parasitoid is highly attracted to cabbage plants infested by *P. brassicae* caterpillars (18) and caterpillar oral secretions are known to contain an elicitor that has the same effect on mechanically damaged cabbage leaves as caterpillar feeding (19, 20). Here we demonstrate that β-glucosidase has the same effect on cabbage plants as regurgitant of the caterpillar *P. brassicae*; it results in the emission of a similar bouquet of volatiles and in the attraction of the parasitoid *C. glomerata*. Moreover, the parasitoids do not discriminate between regurgitant-treated leaves and β-glucosidase-treated leaves. Finally, we show that β-glucosidase is present in caterpillar regurgitant.

MATERIALS AND METHODS

Rearing Procedures. Plants (Brussels sprouts; *Brassica oleracea* L. var. *gemmifera* cv. Titurel), herbivores (*P. brassicae* L., Lepidoptera, Pieridae), and parasitoids (*C. glomerata* L., Hymenoptera, Braconidae) were reared according to a previously described procedure (21).

Bioassay. The behavioral response of individual *C. glomerata* females toward individual leaves of different treatments was observed in an earlier described greenhouse flight chamber setup during a series of dual-choice tests (20, 21). To increase their responsiveness, 4- to 5-day-old female parasitoids were given an experience (20 s) on leaves with host

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: ADold, artificial damage inflicted 1 day before the bioassay; ADfresh, artificial damage inflicted during the bioassay.

†To whom reprint requests should be addressed.

feeding damage 1 day before the experiment (20, 21). Every parasitoid was allowed only one flight attempt.

Enzymes. β-Glucosidase (from almonds; 4–6 units per mg of protein; Sigma) and α-amylase (from *Aspergillus oryzae*; 35 units per mg of solid; Sigma) solutions were applied to cabbage leaves to assay their elicitor activity with the behavioral test. Enzymes were dissolved in 0.1 M NaOH citrate buffer at pH 6 (50 μg/ml). The pH value was the same as that of *P. brassicae* regurgitant.

Plant Treatments. Experiments were conducted with leaves excised from 8-week-old cabbage plants. Leaves were fully expanded and of the same size within each bioassayed combination. Enzyme solutions were applied to leaves that underwent different types of mechanical damage inflicted on the leaves the day before the experiment (day 1) or the day of the experiment (day 2). (*i*) Old artificial damage (ADold): ≈1/3rd of the surface of one leaf was rubbed with 180-grit carborundum powder on a wet cotton wool pad. This damage was inflicted on day 1. (*ii*) Fresh artificial damage (ADfresh): on day 1, undamaged leaves were excised and incubated with their petiole in enzyme solutions or in water. During the flight bioassay, on day 2, one 0.8-cm-diameter hole was punched in the leaf blade with a cork borer every 15 min, starting 1 h before the bioassay (18). (*iii*) Undamaged leaves: leaves were excised on day 1 and incubated with their petiole in enzyme solution or in water. No mechanical damage was inflicted to the leaf blade before or during the bioassay. Treatments of the plants occurred between 3:00 and 4:00 p.m. of day 1, and the flight bioassay was performed between 9:00 and 12:00 a.m. of day 2. Thus, the incubation lasted ≈20 h at 20 ± 2°C, 50–70% relative humidity, scotophase 10:00 p.m. to 6:00 a.m.

Enzyme Application. Enzyme solutions were applied on the different types of artificial damage. The following combinations were tested in the flight chamber: (*i*) βglu on ADold vs. ADold. One leaf treated with 1 ml of β-glucosidase solution applied on carborundum-inflicted artificial damage (βglu on ADold) vs. a control leaf (ADold) where only buffer solution had been applied to the damage. These leaves were excised the next day and used in the bioassay. Two concentrations were tested: 50 and 0.05 μg/ml. (*ii*) βglu_UND vs. UND. One undamaged cabbage leaf was excised and incubated with the petiole for 20 h in 1 ml of β-glucosidase solution at 50 μg/ml (βglu_UND) and tested against a control leaf incubated in citrate buffer (UND). (*iii*) βglu on ADfresh vs. ADfresh. Undamaged leaves were incubated as in the previous bioassay, but during the bioassay ADfresh was inflicted to both test and control leaves. The last two treatments were performed to ascertain whether mechanical damage was necessary for an effect of β-glucosidase on the plant. (*iv*) βglu on ADold vs. REG on ADold. One leaf treated with 25 μl of β-glucosidase solution, containing 0.05 μg of enzyme, applied on ADold (βglu on ADold) vs. a leaf treated with 25 μl of regurgitant obtained from caterpillars fed on Brussels sprouts (21) (REG on ADold). This combination was tested to investigate whether β-glucosidase application and caterpillar regurgitant application can be discriminated by the parasitoids. βglu on ADold (at 0.05 μg of enzyme) was also tested against an ADold leaf (βglu on ADold vs. ADold), in the same experimental day, in order to confirm the attractiveness of the enzyme-treated leaf. (*v*) α-amy on ADold vs. ADold. One milliliter of α-amylase solution, at 50 μg/ml, was applied on the surface of a carborundum-damaged leaf (α-amy on ADold) and tested against a buffer-treated leaf with the same type of damage (ADold) after ≈20 h.

Alternative Treatments. To determine the origin of β-glucosidase in the regurgitant, several bioassays were run. In all these experiments, both test and control leaves were wounded with carborundum 20 h before the bioassay (ADold) and immediately treated. For all combinations the control was an ADold leaf treated with an amount of citrate buffer equivalent to the volume of liquid used to treat the test leaf. (*i*) REG art. diet on ADold vs. ADold. The test leaf was treated with 25 μl of regurgitant obtained from larvae fed on artificial diet (22). (*ii*) Leaf juice on ADold vs. ADold. Leaf juice was obtained by crushing 1 g of cabbage leaf with 750 μl of citrate buffer with a mortar and pestle. The resulting slur was filtered through glass wool and centrifuged at 14,000 × *g* for 30 min, and the supernatant was used. The test leaf was treated with 60 μl or 2 ml of leaf juice. (*iii*) Boiled REG on ADold vs. ADold. This combination was tested to determine whether the elicitor enzyme was rendered ineffective by denaturation. A 50% (vol/vol) solution of regurgitant in buffer was boiled for 10 min and 50 μl was applied on the wounded test leaf.

Enzyme Assay. The presence of β-glucosidase activity was determined in the test materials. Freshly collected samples were assayed in triplicate. Caterpillar head extract was obtained by dissecting the head of 10 fifth-instar caterpillars, which were thoroughly rinsed with citrate buffer to avoid contamination with the gut contents, and crushed with a mortar and pestle in 375 μl of buffer. Artificial diet extract was obtained by crushing 1 g of artificial diet in 1 ml of buffer. In both cases, the slur was filtered and centrifuged as explained for the leaf juice. The incubation mixture contained 5 mM 4-nitrophenyl β-D-glucopyranoside (Boehringer Mannheim) in 1 ml of 0.1 M NaOH citrate buffer (pH 6.0) and either 25 μl of caterpillar regurgitant, 300 μl of head extract, 500 μl of leaf juice, or 500 μl of artificial diet extract. The mixture was briefly stirred on a Vortex and incubated in a water bath at 30°C for 2 h. The reaction was stopped by immersing the incubation tubes in boiling water for 10 min. As a control, an identical mixture was boiled for 10 min before the incubation

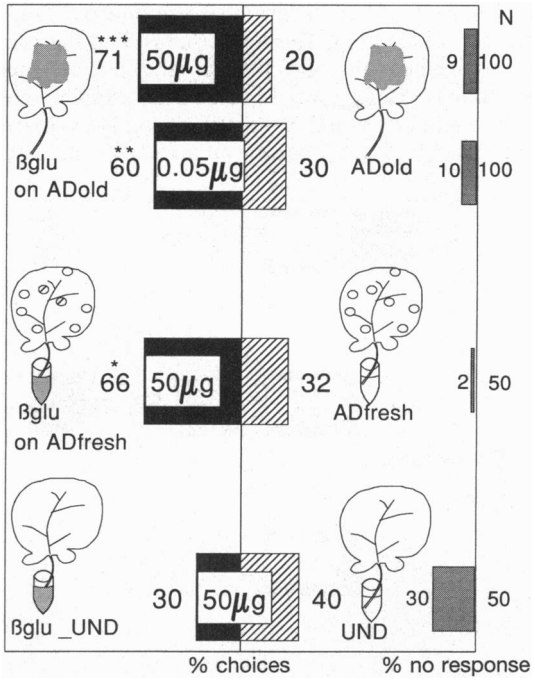

FIG. 1. Flight response of female *C. glomerata* to cabbage leaves treated with β-glucosidase (βglu) (from almonds). Drawings illustrate the type of artificial damage inflicted on the leaves before treatments with enzymes or control solutions. Drawings on the left refer to the left part of the choice bar; drawings on the right refer to the right part of the choice bar. Treatments are indicated below the drawings. Solid bars represent parasitoid choices for the treated leaf and hatched bars represent choices for the control leaf. Number of replicates (N) for every comparison is given on the right. Numbers next to bars indicate percentage of parasitoids making a choice for one of the two odor sources or not making a choice at all. Asterisks indicate significant differences within the choice test: *, $0.01 < P \leq 0.05$; **, $0.001 < P \leq 0.01$; ***, $P \leq 0.001$; χ^2 for goodness-of-fit (23). UND, undamaged.

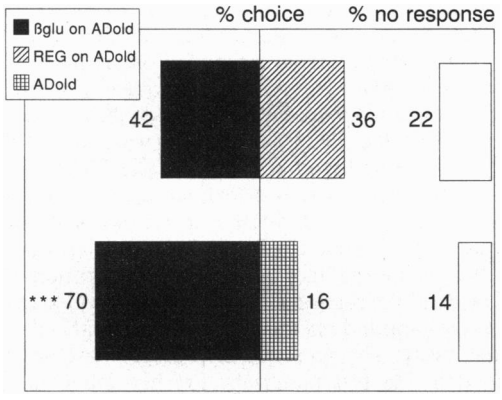

FIG. 2. Flight response of female *C. glomerata* to cabbage leaves treated with almond β-glucosidase (βglu on ADold) compared to leaves treated with caterpillar regurgitant (REG on ADold) with equivalent enzyme activity; $n = 50$ per odor source combination. Other explanations are in Fig. 1 and *Materials and Methods.*

at 30°C. All tubes were centrifuged at 10,000 × *g* for 10 min after incubation and the absorbance of the supernatant was measured in a Zeiss PMQ3 spectrophotometer. The concentration of the reaction product *p*-nitrophenol was determined at 400 nm by using a molar extinction coefficient of 18,130. One unit is defined as the amount of enzyme hydrolyzing 1 μmol of substrate per min at 30°C.

Collection and Analysis of Headspace Volatiles. Volatiles emitted by excised cabbage leaves of different treatments were collected on Tenax-TA traps for 1 h. The adsorbed volatiles were thermally desorbed (Thermodesorption Cold Trap Unit, Chrompack, Middelburg, The Netherlands) and the complete sample was injected into a gas chromatograph/mass spectrometer for analysis (Supelcowax 10 fused silica capillary column; 60 m × 0.25 mm i.d.; film thickness, 0.25 μm; He as carrier gas at 20 cm/s; temperature program, 40°C to 100°C at 2.5°C/min and then to 250°C at 4°C/min) as described (20). Ten cabbage leaves were used per treatment; each treatment was replicated three times. Identical to the bioassays, leaves were used for headspace sampling 20 h after treatment.

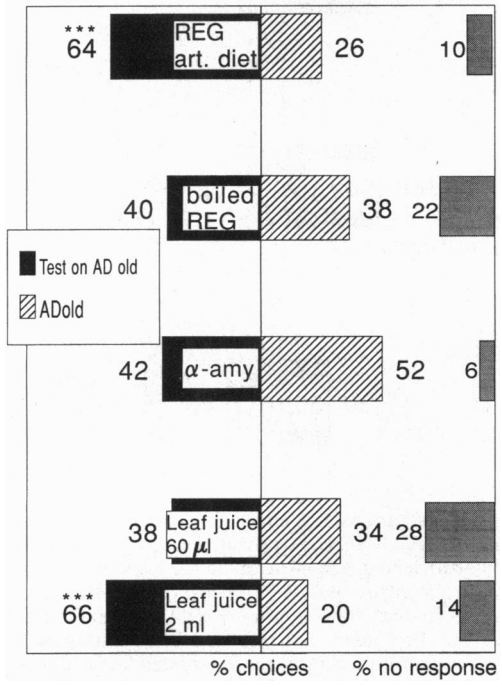

FIG. 3. Flight response of female *C. glomerata* to cabbage leaves treated with different elicitor sources; $n = 50$ per odor source combination. REG art. diet, 25 μl of gut regurgitant collected from caterpillars reared on artificial diet. Other explanations are in Fig. 1 and *Materials and Methods.* α-amy, α-Amylase.

RESULTS

Parasitoid Response. The parasitoids were strongly attracted to mechanically damaged cabbage leaves that were

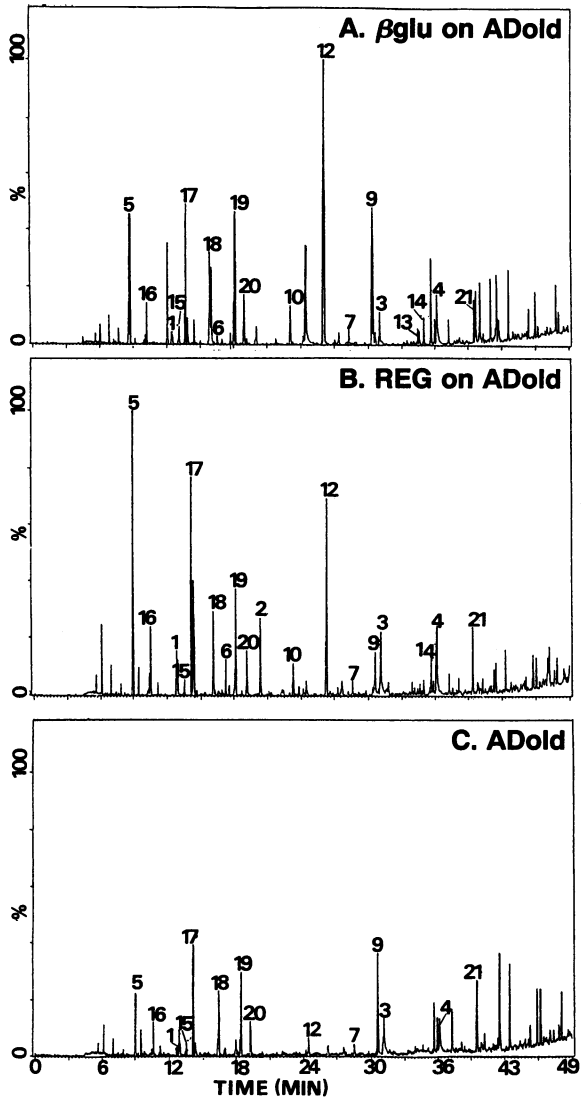

FIG. 4. Total ion chromatograms of headspace collections of cabbage plants that underwent different treatments. Treatments and mean total peak area (brackets) are as follows. (*A*) βglu on ADold, β-glucosidase (from almonds)-treated leaves [56,923]. (*B*) REG on ADold, caterpillar regurgitant-treated leaves [50,475]. (*C*) ADold, artificially damaged leaves [27,887]. Peaks are labeled with a number corresponding to the chemicals identified below. Mean percentages (parentheses) for each compound in treatments *A–C* are listed: 1, hexanal (1–0.7–0.6); 2, (*E*)-2-hexenal (0.5–1.7–0.4); 3, nonanal (1.5–0.9–1.5); 4, decanal (3.3–1–4.3); 5, 3-pentanone (7.3–5.9–4.3); 6, 1-penten-3-ol (1–0.9–2.5); 7, 1-hexanol (1.2–0.9–1.1); 8, (*E*)-2-hexen-1-ol (0.3–0.3–0); 9, (*Z*)-3-hexen-1-ol (9.2–4.3–9.6); 10, 1-hexen-1-yl acetate (4.5–7.7–6.5); 11, (*E*)-2-hexenyl acetate (0.4–1.4–0.6); 12, (*Z*)-3-hexen-1-yl acetate (36.4–39.6–46.2); 13, (*Z*)-3-hexen-1-yl butyrate (1.2–0.4–0.2); 14, (*Z*)-3-hexen-1-yl isovalerate (1.6–0.1–0); 15, β-pinene (0.5–0.6–0.3); 16, α-thujene (1.6–1.3–1.5); 17, sabinene (4.8–7.2–5.3); 18, myrcene (2.9–3.2–2.6); 19, limonene (4.6–6.7–6); 20, 1,8-cineol (1.5–2.3–2); 21, β-elemene (1.6–1.5–2.2). All treatments were made 20 h before collection of headspace volatiles. Unlabeled peaks eluting after peak 21 are system impurities. Other unlabeled peaks are not common to the β-glucosidase and caterpillar regurgitant treatments.

treated with β-glucosidase: 90% of the wasps made a choice and of these the majority chose the β-glucosidase-treated leaf (Fig. 1, βglu on ADold vs. ADold and βglu on ADfresh vs. ADfresh). Application of β-glucosidase through the petiole is effective only when the leaf blade is mechanically damaged (Fig. 1, compare βglu on ADfresh vs. ADfresh and βglu_UND vs. UND). This is similar to the effect of application of caterpillar regurgitant to cabbage leaves (20). The parasitoids are equally attracted to leaves treated with either larval regurgitant or almond β-glucosidase, applied in amounts with equivalent enzyme activity (see below) (Fig. 2). A strong attraction was also observed to leaves treated with regurgitant from caterpillars fed an artificial diet (Fig. 3). Application of boiled regurgitant or the enzyme α-amylase onto mechanical damage did not yield attraction of the parasitoids (Fig. 3). Application of leaf juice onto mechanical damage resulted in parasitoid attraction when 2 ml of the extract was applied. However, leaves treated with 60 μl of leaf juice, which has a β-glucosidase activity equivalent to 25 μl of regurgitant (see below), were not preferred over mechanically damaged leaves (Fig. 3).

Presence of β-Glucosidase in Treatment Materials. β-Glucosidase was detected in *P. brassicae* regurgitant with an activity of 0.074 ± 0.012 unit per 25 μl (mean ± SD; $n = 3$) with 4-nitrophenyl β-D-glucopyranoside as substrate. The amounts of β-glucosidase were determined with a standard curve based on different concentrations of almond β-glucosidase. The amount of β-glucosidase in 25 μl of regurgitant (obtained from ≈5 larvae) is equivalent to 0.01–0.05 μg of almond β-glucosidase. Application of this amount of almond β-glucosidase onto mechanical damage clearly results in attraction of the parasitoids (Fig. 1). β-Glucosidase activity was also detected in caterpillar head extract (0.017 ± 0.001 unit per larva), in the regurgitant of artificial diet-fed larvae (0.0046 ± 0.0004 unit per larva equivalent), and in the cabbage leaf juice (0.62 ± 0.06 unit/g). No β-glucosidase activity was detected in an extract of artificial diet.

Chemical Analysis of Enzyme-Induced Plant Volatiles. Cabbage leaves treated with β-glucosidase release a volatile blend that is very similar to the blend emitted by regurgitant-treated cabbage leaves (Fig. 4), while artificially damaged leaves (ADold) produced a lower amount of volatiles. A quantitative analysis was made for those components that were also present in the headspace of cabbage leaves treated with caterpillar regurgitant (20). These compounds are assumed to be involved in attraction of the parasitoids. For each compound, the mean peak area was divided by the mean peak area in the undamaged treatment. When a chemical was not present in the undamaged treatment, a peak area of 1 was given in order to avoid division by 0. This transformation was performed for 21 compounds that comprised 90–95% of the total peak area of the 49 identified compounds. The analysis clearly shows that the β-glucosidase treatment results in a bouquet that is very similar to that of regurgitant-treated leaves (Fig. 5), and this blend is quantitatively different from leaves that were mechanically damaged only (ADold) and not enzymatically treated.

DISCUSSION

β-Glucosidase is present in *P. brassicae* regurgitant. Application of the enzyme onto mechanically damaged cabbage leaves results in the emission of a blend of volatiles that is similar to that emitted by regurgitant-treated leaves and also in attraction of the parasitoid *C. glomerata*. The effects of β-glucosidase and caterpillar regurgitant are apparent under similar conditions. Incubation of undamaged cabbage leaves in a solution of β-glucosidase results only in the emission of parasitoid-attracting volatiles when the leaf surface is damaged. This was also recorded for leaves incubated in a regurgitant solution (20). A spatial separation of the enzyme and its substrate might explain the need of mechanical wounding for an effect. β-Glucosidase might be transported only in extracellular spaces, with the substrate becoming available upon fragmen-

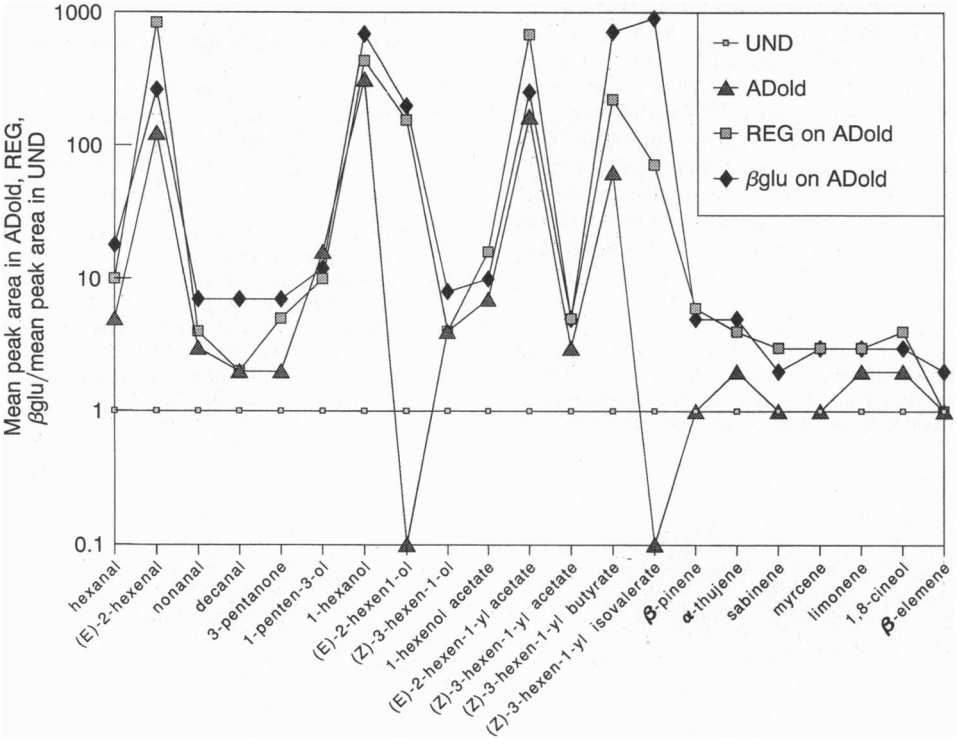

FIG. 5. Relative increase of selected compounds (x axis) common to the caterpillar regurgitant (REG) and β-glucosidase (βglu) treatments calculated proportional to the amounts emitted by undamaged leaves (UND). Values of y axis (logarithmic scale) indicate the ratio between the average peak area of every compound in the βglu, REG, and ADold treatment and the average area of the same peak in the UND treatment.

tation of the cell wall. Several models have been developed for pathogen-related defense responses to explain such a phenomenon (24–26). This requirement of mechanical damage for the emission of herbivore-induced volatiles has not been reported for other plants. Corn leaves that are incubated in a caterpillar regurgitant solution or lima bean leaves that are incubated in a solution of endogenous systemic elicitor emit carnivore attractants without any mechanical damage to the leaf blade (14, 27).

β-Glucosidase is also present in crushed cabbage leaves, but only large amounts of leaf juice, with high plant β-glucosidase activity, resulted in parasitoid attraction. This indicates that cabbage β-glucosidase is different from *P. brassicae* β-glucosidase. The caterpillars, or associated microorganisms, produce β-glucosidase themselves, because when they feed on a diet that does not contain detectable β-glucosidase, their regurgitant contains β-glucosidase and induces the emission of carnivore attractants. The enzyme is presumably produced in the mouth area of *P. brassicae* caterpillars, since β-glucosidase activity of head extract of one larva (0.017 unit) is similar to the activity of the regurgitant obtained from one larva (5 μl of regurgitant = 0.014 unit). Thus, β-glucosidase is an elicitor secreted by the herbivore that induces the response of cabbage plants to *P. brassicae* caterpillars.

Some signals involved in the induction of the defense of plants to pathogens and herbivores occur in the plant as β-glucosides (25, 28). Inactive signals, conjugated as glucosides, are abundant in plant tissues and their physiological activity correlates with their hydrolysis rate (29). Boland *et al.* (15) suggested that the homoterpene carnivore attractants emitted by spider mite-infested lima bean leaves (12) are stored as β-glucosides. They showed that lima bean plants emit the two homoterpenes that are produced in response to spider mite infestation (12) when β-glucosidase is applied into a mechanical wound, although the effect is less pronounced than that of spider mite infestation (15). In our study, however, β-glucosidase has an effect that is similar to that of herbivore damage. Moreover, it is remarkable that we observed such a similarity in both the phytochemical and the behavioral response when we used a β-glucosidase not produced by caterpillars but obtained from almonds. On the other hand, almond β-glucosidase is known to be a mixture of β-glucosidases with a broad degree of substrate specificity (30). Furthermore, we detected β-glucosidase activity in human saliva (probably from microorganisms) and we observed that damaged cabbage leaves treated with human saliva exerted a significant attraction on the parasitoids over mechanically damaged leaves (data not shown). These observations suggest that a nonspecific β-glucosidase is sufficient to induce emission of carnivore attractants by cabbage.

We thank H. Snellen, A. Gidding, and L. Koopman for rearing of insects; A. B. Koopmanschap-Memelink, P. E. M. Martin, A. Vermeesch, and A. Vermunt for advice on enzyme assay procedures; and C. Bolter, J. J. A. van Loon, R. de Jong, L. M. Schoonhoven, J. H. Tumlinson, T. C. J. Turlings, L. E. M. Vet, and P. J. G. M. de Wit for valuable comments on a previous version of the manuscript. We thank the Dutch Organisation for Scientific Research (NWO) and the University of Perugia, Italy, for financial support.

1. Tallamy, D. W. & Raupp, M. J., eds. (1991) *Phytochemical Induction by Herbivores* (Wiley, New York).
2. Dicke, M. (1994) *J. Plant Physiol.* **143,** 465–472.
3. Dixon, R. A. (1986) *Biol. Rev.* **61,** 239–291.
4. Ryan, C. A. & Farmer, E. E. (1991) *Annu. Rev. Plant Physiol. Plant. Mol. Biol.* **42,** 651–743.
5. Miles, P. W. (1969) *Entomol. Exp. Appl.* **12,** 736–744.
6. Lin, H., Kogan, M. & Fischer, D. (1990) *Environ. Entomol.* **19,** 1852–1857.
7. Hartley, S. E. & Lawton, J. H. (1991) in *Phytochemical Induction by Herbivores*, eds. Tallamy, D. W. & Raupp, M. J. (Wiley, New York), pp. 105–132.
8. Farmer, E. E. & Ryan, C. A. (1992) *Plant Cell* **4,** 129–134.
9. Enyedi, A. J., Yalpani, N., Silverman, P. & Raskin, I. (1992) *Cell* **70,** 879–886.
10. Dicke, M. & Sabelis, M. W. (1988) *Neth. J. Zool.* **38,** 148–165.
11. Dicke, M., Sabelis, M. A., Takabayashi, J., Bruin, J. & Posthumus, M. A. (1990) *J. Chem. Ecol.* **16,** 3091–3118.
12. Dicke, M., van Beek, T. A., Posthumus, M. A., Ben Dom, N., van Bokhoven, H. & De Groot, A. E. (1990) *J. Chem. Ecol.* **16,** 381–396.
13. Turlings, T. C. J., Tumlinson, J. H. & Lewis, W. J. (1990) *Science* **250,** 1251–1253.
14. Turlings, T. C. J., McCall, P. J., Alborn, H. T. & Tumlinson, J. H. (1993) *J. Chem. Ecol.* **19,** 411–425.
15. Boland, W., Feng, Z., Donath, J. & Gäbler, A. (1992) *Naturwissenschaften* **79,** 368–371.
16. Yu, S. J. (1989) *Insect Biochem.* **19,** 103–108.
17. Ahmad, S. A. & Hopkins, T. L. (1992) *Arch. Insect. Biochem. Physiol.* **21,** 207–224.
18. Steinberg, S., Dicke, M. & Vet, L. E. M. (1993) *J. Chem. Ecol.* **19,** 47–60.
19. Sato, Y. (1979) *Physiol. Entomol.* **4,** 63–70.
20. Mattiacci, L., Dicke, M. & Posthumus, M. A. (1994) *J. Chem. Ecol.* **20,** 2229–2247.
21. Steinberg, S., Dicke, M., Vet, L. E. M. & Wanningen, R. (1992) *Entomol. Exp. Appl.* **63,** 163–175.
22. David, W. A. L. & Gardiner, B. O. C. (1981) *Bull. Entomol. Res.* **56,** 581–593.
23. Sokal, R. R. & Rohlf, F. J. (1981) *Biometry* (Freeman, New York).
24. Conn, E. E. (1984) *Annu. Proc. Phytochem. Soc. Eur.* **24,** 1–28.
25. Hennig, J., Malamy, J., Grynkiewicz, G., Indulski, J. & Klessig, D. F. (1993) *Plant J.* **4,** 593–600.
26. Bacic, A., Harris, P. J. & Stone, B. A. (1988) in *The Biochemistry of Plants,* ed. Conn, E. E. (Academic, New York), Vol. 14, pp. 297–371.
27. Dicke, M., van Baarlen, P., Wessels, R. & Dijkman, H. (1993) *J. Chem. Ecol.* **19,** 581–599.
28. Sembdner, G. & Parthier, B. (1993) *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **44,** 569–589.
29. Hösel, W. (1981) in *The Biochemistry of Plants,* ed. Conn, E. E. (Academic, New York), Vol. 7, pp. 725–751.
30. Conn, E. E. (1993) in *β-Glucosidases: Biochemistry and Molecular Biology,* ed. Esen, A. (Am. Chem. Soc., Washington, DC), pp. 15–26.